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Research article

Environmental influences on the gametic investment of yellow dung fly males

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Abstract. The energetic investment per spermatozoon and in spermatogenesis is central to a male's reproductive strategy. Relatively little, however, is known about environmental influences on variation in male allocation decisions and associated trade-offs. Plasticity in sperm length and testis size in response to variable food and temperature conditions either before or after adult eclosion was investigated in *Scathophaga stercoraria*, a classic model organism for sperm competition. Both measures showed interesting and clear environmental effects and also a heritable component. Testis length, and thus presumably sperm production, showed a hypoallometric ($b < 1$), but non-linear increase with body size, indicating that the allometric relationship changed with size. Like body size, testis length decreased with increasing developmental temperatures, but also showed a complex cubic relationship with adult temperatures. In contrast, sperm length increased or showed a negative quadratic relationship with increasing temperatures. The increase of within-male variation in sperm length with increasing developmental temperature and decreasing adult food indicates that some of our treatments were stressful. Nevertheless, there was no evidence of a trade-off between testis size and sperm length. The missing effect of adult or larval food availability on testis and sperm length, despite strong effects of larval food on body size, suggests that investment into reproduction is less sensitive to food restriction than investment into growth.

Key words: food and temperature stress, life history trade-off, *Scathophaga stercoraria*, sperm competition, sperm length

Introduction

Gametic investment consists of the energy invested into gamete production as a whole and per individual gamete. As males usually produce small gametes, they are predisposed to compete by sheer numbers rather than size or quality of sperm (Parker, 1982). The importance of sperm size, however, is generally unknown. It is therefore unclear whether a correlation and especially a trade-off between sperm size and number is to be expected (e.g. Briskie *et al.*, 1997). Theory predicts that sperm size is optimized independent of sperm competition risk (Parker, 1993) and there is, for instance, evidence for a positive association

with female reproductive tract dimensions (Morrow and Gage 2001a). If long sperm were superior in competition and not more costly (but see Pitnick *et al*., 1995; Pitnick, 1996), number and size could increase simultaneously, as in sneaker males of the dung beetle *Onthopagus binodis* (Simmons *et al*., 1999). In comparative studies high sperm competition risk indeed is commonly associated with increased sperm numbers and/or energetic investment into testes (e.g. Gage, 1994, 1998; Pitnick, 1996; Stockley *et al*., 1997; Wedell, 1997). In contrast, sperm length increases (Morrow and Gage, 2000), decreases (Stockley *et al*., 1997) or is unrelated to sperm competition risk (Briskie *et al*., 1997; Hosken, 1997) among species. There is also no clear pattern within species (Gage and Cook, 1994; Pitnick and Markov, 1994; Gage *et al*., 1998; Hosken *et al*., 2001; Pitnick *et al*., 2001), except for an increase in dung beetles (Simmons *et al*., 1999). Only one study demonstrates an intra-specific size/number trade-off, for non-fertilizing oligopyrene sperm in a snail (Oppliger *et al*., 1998).

Inter-individual variation in sperm size is considerable in several species and equally enigmatic (Ward, 1998). Larger sperm may have a competitive advantage if they swim faster, fill a female's sperm stores more quickly, survive longer within or are preferred by females, ultimately conferring higher fertilization success (Katz and Drobnis, 1990; Gomendio and Roldan, 1991; Briskie and Montgomerie, 1992; Gage, 1994; Pitnick and Markov, 1994; Bressac and Hauschteck-Jungen, 1996; Stockley *et al*., 1997). Similar arguments could be made for smaller sperm (e.g. Ghiselin, 1974). To date there is some, but by no means conclusive evidence for sperm length specific fertilization success within species (Radwan, 1996; Gage *et al*., 1998; LaMunyon and Ward, 1998; Snook and Karr, 1998; but see Morrow and Gage, 2001b). Sperm length and body size are typically uncorrelated within species (Ward and Hauschteck-Jungen, 1993; Pitnick and Markov, 1994; Gage *et al*., 1998), and either positively (Gage, 1994; Pitnick *et al*., 1995; Briskie *et al*., 1997) or not correlated (Harcourt, 1991; Hosken, 1997; Gage, 1998) among species. In contrast, testis size, and thus presumably sperm number, generally increases with body size within and among species (Ward and Simmons, 1991; Pitnick and Markov, 1994; Hosken, 1997; Gage, 1998). In the few species examined, high heritabilities more typical of traits less connected to fitness (cf. Mousseau and Roff, 1987) were found for sperm size (Beatty, 1970; Ward, 2000; Morrow and Gage, 2001c) and testis size (Pitnick and Miller, 2000). In summary, there is rather inconsistent evidence for adaptive variation in sperm size.

The intra-specific functional significance of sperm size and a possible size/number trade-off are rarely investigated directly (but see Oppliger *et al*., 1998), probably primarily because of methodological difficulties, such as how to reliably determine absolute sperm numbers produced (cf. Simmons *et al*., 1999). An indirect approach to these questions is to estimate sperm production via testes size (e.g. Simmons *et al*., 1999; Hosken and Ward, 2001; Stockley and

Seal, 2001; but see Pitnick, 1996) to study size variation of sperm and testes, i.e. gametic investment, in response to environmental influences (Gage and Cook, 1994; Stockley and Seal, 2001). Food availability and temperature are two key factors influencing female reproductive strategies and (animal) life histories in general (Stearns, 1992). Nutrient limitation during the juvenile or adult life stage affects most fitness components (e.g. Blanckenhorn, 1998a, 2000), and very likely also testis development, sperm number and/or quality (Gage and Cook, 1994; Droney, 1998). Temperature is similarly effective. In ectotherms high temperatures generally reduce body, cell and egg size (Atkinson, 1994; Partridge *et al.*, 1994; Van Voorhies, 1996). Temperature effects on sperm size have not yet been studied.

Here we investigate how males allocate resources to sperm production in the yellow dung fly, *Scathophaga stercoraria* (L.), a classic model for sperm competition (e.g. Parker, 1970; Simmons and Parker, 1992; Ward, 1993). Females mate multiply and males struggle for mates and copulate forcefully. Fly gonads start developing before adult eclosion (Kerkis, 1931), and sperm production continuously proceeds thereafter (unlike in Lepidoptera: see Gage and Cook, 1994). Testis size is positively correlated with body size (Ward and Simmons, 1991) and responds to selection via enforced polyandry (Hosken and Ward, 2001). Sperm length varies extensively among males (Ward and Hauschteck-Jungen, 1993), is highly heritable (Ward, 2000) and can serve as a marker of male identity (e.g. Hellriegel and Bernasconi, 2000).

We experimentally manipulated two key environmental factors at two life stages and studied their effects on male investment in testes (i.e. maximum sperm number) relative to individual sperm (cf. Gage and Cook, 1994; Stockley and Seal, 2001). We varied temperature and food availability from normal to stressful levels either before or after adult eclosion and measured the responses of sperm length and testes length. For males developing under stressfully high temperatures and limited nutrients we predicted a correlated reduction in body and testes size (Blanckenhorn, 1997, 1998a; but see Gage, 1995; Stockley and Seal, 2001). Based on findings in prey-deprived adults (Foster, 1967) we also expected smaller testes when food was limited after emergence. Because of the importance of relative sperm numbers in *S. stercoraria* (Simmons and Parker, 1992), we also expected corresponding decreases in sperm length to compensate for the predicted stress-induced testis size reduction, assuming a trade-off between sperm size and number.

Materials and methods

Three experiments were performed in the laboratory (see below). All flies originated from our study population in Fehraltorf near Zurich and had been

reared and held in the laboratory using standard methods (Blanckenhorn, 1997) for at least one generation. Yellow dung fly larvae develop and live in cow dung. At more than 2 g dung per larva, competition has no noticeable effect on pre-adult survival or adult size, whereas less than 1 g dung reduces both parameters (Amano, 1983; Blanckenhorn, 1998a). Adults can subsist on sugar and water but require prey to produce eggs and sperm (Foster, 1967; Gibbons, 1980). *Drosophila melanogaster* was used as prey. Yellow dung flies prefer, and are adapted to, cooler temperatures between 10 and 20 °C (Blanckenhorn, 1997). Temperatures below 10 °C are likely to induce pupal diapause (Blanckenhorn, 1998b). Temperatures beyond 22 °C are probably stressful and those beyond 25 °C tend to kill larvae, pupae and adults (Ward and Simmons, 1990; Blanckenhorn, 1998a).

Pre-adult treatments before adult eclosion

To assess the effect of (1) dung availability and (2) rearing temperature on sperm length and testes size, and to estimate the allometric relationship between body and testes size, flies were reared at different temperatures, 13 h photoperiod and two larval densities. We chose to alter larval density by varying the mean amount of dung available to each larva, as opposed to changing the number of larvae in a set amount of dung (e.g. Stockley and Seal, 2001). Two similar experiments were carried out, because at first we measured sperm but not testis length. The first experiment included two (intermediate and high) and the second experiment three (low, intermediate and high) temperatures (see below).

Laboratory-reared females were allowed to copulate with a male and lay eggs on a portion of dung, the larval resource. To generate the test flies, their full clutches of 50–90 eggs were subdivided into four approximately equal-sized subportions in the first experiment, and six subportions in the second. One half of the subportions (two or three respectively) was then allowed to develop in a 50 ml plastic container with more than 2 g and the other half with less than 1 g of defrosted, uniform cow dung per individual. One container per dung treatment was reared at 15 and 23 °C in the first experiment, and 12, 18 and 24 °C in the second experiment. Thus, offspring from each female (henceforth referred to as a family) experienced all four or six pre-adult treatment combinations. Using brothers minimized genetic variation in the traits of interest among treatment combinations. It also allowed the testing of variation among families ($n = 18$ in experiment 1, $n = 16$ in experiment 2).

After emergence, one to three randomly chosen males from each family within each treatment combination was held singly in a 100 ml bottle ($n = 17$–30 males per treatment combination). Adults were supplied with *ad libitum*

sugar, water, and *D. melanogaster*. Males from all pre-adult treatments (i.e. $n_{total} = 90$ males in first, $n_{total} = 137$ in second experiment) were held as adults at the same constant 19 °C, 60% r.h. and 13 h photoperiod and frozen after 14 days. Two weeks is sufficient time for males to develop sperm and fill their testes (Foster, 1967; Henseler, 1998). Subsequently the length of one of their hind tibiae, one of their testes and 30 of their sperm was measured (details below).

Treatments after adult eclosion

To assess the effect of adult holding temperature and food availability on testis and sperm length, adult males were held at four temperatures and two food regimes. To generate the test flies, partial clutches of 10–20 eggs from laboratory-reared females (i.e. $n = 38$ families) were allowed to hatch and develop in 50 ml plastic containers with more than 2 g per individual of defrosted, uniform dung at 19 °C, 60% r.h. and 13 h photoperiod. Variation in body size and its effects on testes and sperm size were thus minimized (Amano, 1983, see above). Upon emergence, males were held singly in 100 ml bottles ($n_{total} = 122$). Four males from each clutch were randomly assigned to climate chambers at 60% r.h., 13 h photoperiod and either 11, 15, 19 or 23 °C ($n = 14$–16 males per treatment combination). Using brothers again minimized genetic variation in the traits of interest among temperature and food treatments. The males were supplied with *ad libitum* sugar and water, and *D. melanogaster* either *ad libitum* or a limited 10 per week (cf. Jann and Ward, 1999). After 14 days, the males were frozen and measured as described below.

Measurement of body size, testis size and sperm length

For each male, hind tibia length was measured (to the nearest μm at × 16 magnification) as a reliable measure of male body size (e.g. Ward and Simmons, 1991). After defrosting males were dissected, their two testes removed and singly transferred to a slide with insect ringer. After measuring testis length (as in Ward and Simmons, 1991) the sperm from the third of the testis proximal to the ejaculatory duct was released. We spread out the sperm, dried the slide, and then washed it to remove salt crystals. The length of 30 sperm per male from one randomly chosen testis was measured using the Optimas© image analysis software (16 × 25 magnification). The measurer traced the entire length (head and tail) of a randomly chosen intact sperm with the mouse, and a pre-programmed algorithm tracked and smoothed his/her movements. To estimate the accuracy of each measurer, 30 individual sperm from eight randomly chosen males were measured again (blind) by the same person after several

days. For each of the three experiments sperm length was measured by a different person.

Statistical analysis

Though temperature is a continuous variable we here treated it as discrete because we manipulated it. Therefore, an ANCOVA approach was used, followed by a test for linear, quadratic and cubic trends (trend analysis or polynomial contrasts, see Tabachnik and Fidell, 1989). Each factorial ANOVA included family of origin, food and temperature treatment as fixed factors and hind tibia length as a covariate and was calculated using SPSS 6.1.1 for Macintosh (SPSS, 1995). Using hind tibia length as a covariate accounted for possible correlations with, and therefore revealed treatment effects not explained by, body size. Family was treated as a blocking factor, so no interaction with this variable entered the models to start with. When other interactions were not significant ($p > 0.2$), implying additivity of the effects of the main factors, we removed them from the model. Some formal outliers (>3.5 SD from mean) were removed prior to analyses as indicated in Tables 1–3.

Table 1. Hind tibia length, mean and variation SD in sperm length of males treated before adult eclosion in experiment 1 (mean ± SE)

Temperature (°C)	Food level	Tibia length (mm)	Sperm length (μm)	SD in sperm length	*n*
15	Low	3.83 ± 0.03	203.8 ± 0.9	3.2 ± 0.2	17
	High	3.96 ± 0.02	205.3 ± 0.8	3.1 ± 0.2	25
23	Low	3.66 ± 0.02	207.4 ± 1.2	3.9 ± 0.2	27[a]
	High	3.77 ± 0.02	207.5 ± 0.9	3.9 ± 0.2	30

[a] One formal outlier (>3.5 SD from mean) was removed in the analysis.

Table 2. Hind tibia length, mean testis length and mean and variation SD in sperm length of males treated before adult eclosion in experiment 2 (mean ± SE)

Temperature (°C)	Food level	Tibia length (mm)	Testis length (mm)	Sperm length (μm)	SD in sperm length	*n*
12	Low	3.10 ± 0.07	1.78 ± 0.03	209.0 ± 0.8	4.0 ± 0.2	21
	High	3.65 ± 0.04	1.99 ± 0.04	209.8 ± 0.9	3.5 ± 0.2	21
18	Low	3.10 ± 0.05	1.69 ± 0.03	210.9 ± 1.1	3.8 ± 0.4	24
	High	3.61 ± 0.03	1.88 ± 0.04	212.5 ± 0.9	4.1 ± 0.4	24
24	Low	2.97 ± 0.06	1.55 ± 0.03	209.4 ± 0.9	4.7 ± 0.3	25[a]
	High	3.44 ± 0.06	1.65 ± 0.03	211.1 ± 1.1	5.0 ± 0.3	211

[a] One formal outlier (>3.5 SD from mean) was removed in the analysis.

Table 3. Hind tibia length, mean testis length and mean and variation SD in sperm length of males treated after adult eclosion (mean ± SE)

Temperature (°C)	Food level	Tibia length (mm)	Testis length (mm)	Sperm length (μm)	SD in sperm length	*n*
11	Low	3.91 ± 0.04	1.95 ± 0.04	201.5 ± 1.5	2.9 ± 0.1	16
	High	3.85 ± 0.02	1.81 ± 0.07	202.2 ± 0.8	2.7 ± 0.1	16
15	Low	3.90 ± 0.04	1.83 ± 0.05	202.0 ± 1.0	3.3 ± 0.2	14
	High	3.91 ± 0.03	1.81 ± 0.07	201.8 ± 1.2	2.9 ± 0.2	14
19	Low	3.94 ± 0.04	1.99 ± 0.05	201.4 ± 0.7	3.2 ± 0.3	13**
	High	3.87 ± 0.04	2.09 ± 0.07	203.8 ± 0.9	2.7 ± 0.1	15
23	Low	3.86 ± 0.04	1.85 ± 0.09	203.3 ± 0.7	3.2 ± 0.2	16
	High	3.92 ± 0.03	1.93 ± 0.04	203.9 ± 0.9	2.9 ± 0.2	15*

* One and ** two formal outliers (>3.5 SD from mean) were removed in the analysis.

Results

Effect of treatments before adult eclosion

Body size

Dung availability (i.e. larval density) and temperature conditions before eclosion both affected final body size as expected (Blanckenhorn, 1997, 1998a): high dung availability and low temperatures resulted in larger adult males (effect of dung availability: $F_{1,131} = 141.52$, $p < 0.001$; temperature: $F_{2,131} = 6.02$, $p < 0.003$; Tables 1 and 2).

Testis length

In the second experiment, temperature significantly influenced testis length ($F_{2,94} = 21.11$, $p < 0.001$): low temperatures resulted in larger testes (Fig. 1b right and Table 2). As expected for a morphological trait, testis size was positively correlated with body size (Fig. 2a; partial $r = 0.52$, $t_{94} = 5.16$, $p < 0.001$; cf. Ward and Simmons, 1991). The quadratic fit yielded a slightly greater R^2 (Fig. 2a), and in a multiple stepwise regression only the quadratic term remained, indicating that the allometric relationship between testes and body size changed with male size. Interestingly, dung availability (i.e. larval competition) had no independent effect on testis size once its effects on body size were removed ($F_{1,94} = 0.05$, $p = 0.83$; Fig. 1b, inset). In addition, there was a significant family effect ($F_{37,94} = 1.97$, $p = 0.005$) indicating a heritable component of testis size.

Sperm length

In both experiments, rearing temperature before eclosion affected sperm length whereas dung availability did not. In the first experiment, lower temperatures

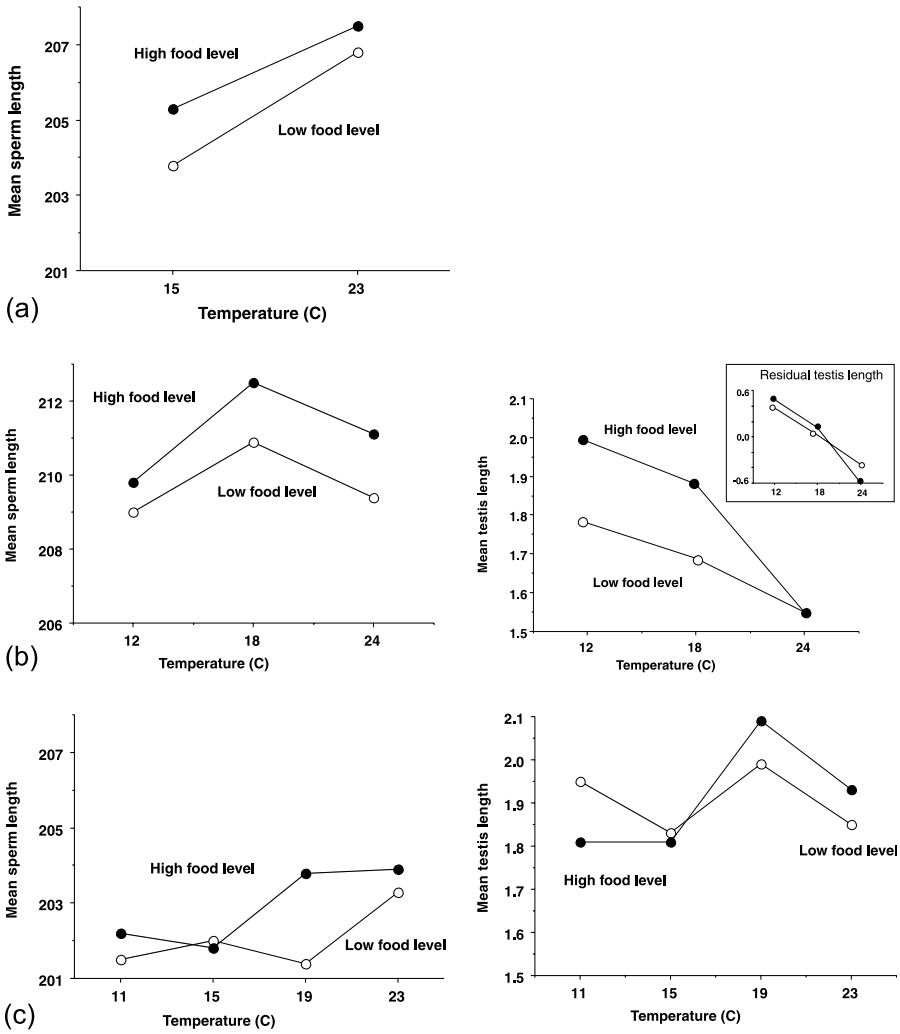

Figure 1. Influences of temperature and food availability on mean sperm length and mean testis size of 14 day old yellow dung flies treated differently before adult eclosion (i.e. as larvae and pupae) (a) in experiment 1 (where only sperm length was measured), (b) in experiment 2 and (c) after adult eclosion as pre-reproductive adults. The inset in (b) shows the residuals of mean testis length after removal of the effect of hind tibia length. Symbols: open dots indicate low and filled dots high food levels.

resulted in shorter sperm ($F_{1,78} = 6.57$, $p = 0.012$; Fig. 1a and Table 1). Dung availability had no effect on sperm length ($F_{1,78} = 0.85$, $p = 0.36$), nor did hind tibia length, as expected (partial $r = 0.03$, $t_{78} = 0.2$, $p = 0.84$; cf. Ward and Hauschteck-Jungen, 1993). There was a family effect ($F_{17,78} = 4.54$, $p < 0.001$) reflecting the high heritability of sperm length (Ward, 2000). Analysis of the standard deviation (SD) in sperm length within males gave the same picture:

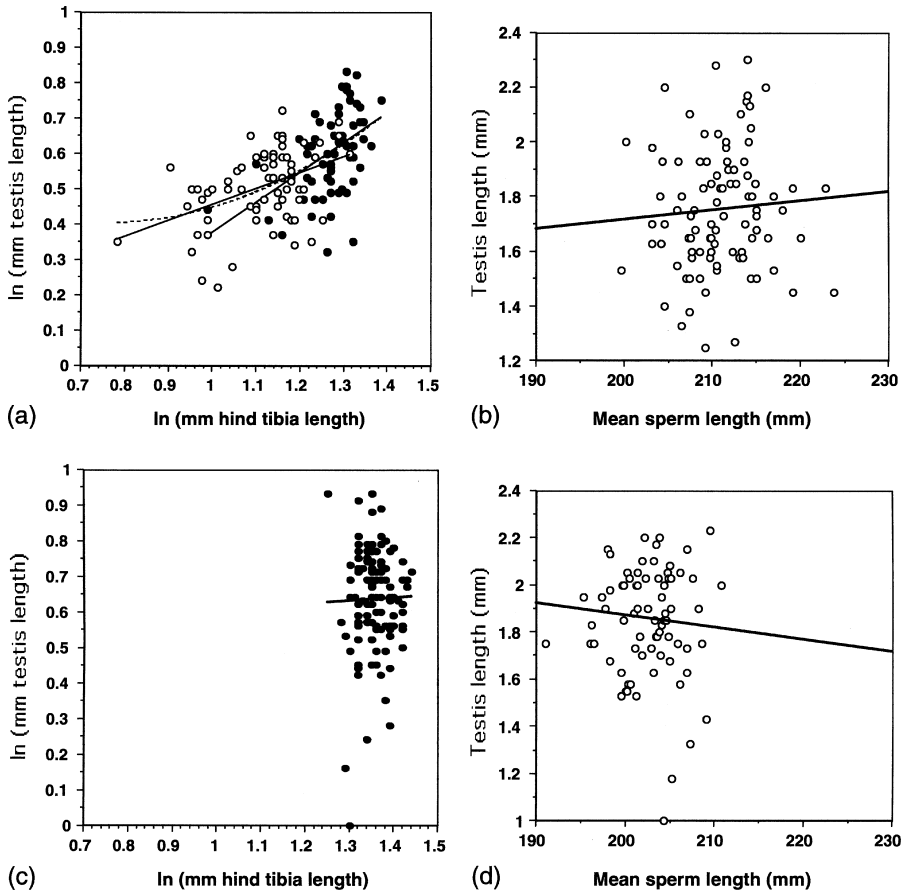

Figure 2. Testis length vs. hind tibia length (a, c) or sperm length (b, d) for 14 day old yellow dung flies treated differently (a), (b) before adult eclosion (i.e. as larvae and pupae) in experiment 2 and (c), (d) after adult eclosion as pre-reproductive adults. (a) Quadratic fit: $Y = 0.78X^2 - 1.19X + 0.86$ ($R^2 = 0.32$); low food and all temperatures (white dots): $Y = 0.45X + 0.001$ ($R^2 = 0.18$); high food and all temperatures (black dots): $Y = 0.84X - 0.47$ ($R^2 = 0.21$). (b) $Y = 0.003X + 1.06$ ($R^2 = 0.01$). (c) $Y = -0.015X + 1.97$ ($R^2 = 0.00$). (d) $Y = -0.005X + 2.92$ ($R^2 = 0.0.01$). Note that (a) and (c) use the same range of X-values to ease the comparison of size ranges between experiments.

SD increased with temperature and varied among families (temperature: $F_{1,78} = 13.83$, $p < 0.001$; family: $F_{17,78} = 2.95$, $p = 0.001$) but not with dung availability and hind tibia length (food: $F_{1,78} = 0.26$, $p = 0.61$; hind tibia length: partial $r = 0.04$, $t_{78} = 0.32$, $p = 0.75$; Table 1).

In the second experiment, developmental temperature also affected sperm length ($F_{2,93} = 3.23$, $p = 0.044$). Subsequent trend analysis revealed a significant negative quadratic ($F_{1,93} = 6.5$, $p = 0.013$), but no linear ($F_{1,93} = 0.1$, $p = 0.78$), relationship between temperature and sperm length (Fig. 1b left and

Table 2). Again, there was significant variation among families ($F_{37,93} = 2.93$, $p < 0.001$), whereas dung availability ($F_{1,93} = 0.17$, $p = 0.68$), hind tibia and testis length (a further covariate) did not affect sperm length (hind tibia length: partial $r = 0.02$, $t_{93} = 0.16$, $p = 0.88$; testis length: partial $r = -0.10$, $t_{93} = -0.79$, $p = 0.43$). The lack of effect of testis size indicates no trade-off between sperm length and testis length within treatment combinations. There was also no evidence of a trade-off when pooling all data and treating them as if they were a phenotypic sample (Fig. 2b; partial $r = -0.04$, $t_{133} = -0.56$, $p = 0.58$; effect of tibia length removed). Analysis of SD in sperm length within males revealed an increasing effect of temperature ($F_{2,91} = 8.44$, $p < 0.001$; Table 2), no effect of dung availability ($F_{1,91} = 0.03$, $p = 0.86$) and, unlike in the first experiment, only a marginal family effect ($F_{37,91} = 1.42$, $p = 0.089$). Interestingly, SD in sperm length increased with hind tibia length (partial $r = 0.35$, $t_{91} = 2.16$, $p = 0.033$) but was unaffected by testis length (partial $r = -0.08$, $t_{91} = -0.52$, $p = 0.60$).

Effect of treatments after adult eclosion

Testis length

Temperature conditions after eclosion significantly affected testis length ($F_{3,95} = 5.63$, $p < 0.001$; Fig. 1c right and Table 3), and trend analysis revealed a cubic ($F_{1,95} = 13.10$, $p < 0.001$), but no linear ($F_{1,95} = 2.22$, $p = 0.14$) or quadratic ($F_{1,95} = 2.02$, $p = 0.16$), relationship between temperature and testis length. Food availability after eclosion had no effect ($F_{1,95} = 0.01$, $p = 0.98$), but the interaction between food and temperature treatment was close to being significant (Fig. 1c): the relationship between testis length and temperature increased more steeply at high food levels ($F_{2,95} = 2.51$, $p = 0.064$). Again, there was significant variation among families ($F_{15,95} = 4.33$, $p < 0.001$) indicating a heritable component of testis size. Finally, testis size was not correlated with hind tibia length (Fig. 2c; partial $r = -0.15$, $t_{95} = -1.68$, $p = 0.097$). This is likely due to the fact that all males had experienced the same conditions before eclosion and therefore covered a rather narrow range of body sizes (Table 1 and Fig. 2c).

Sperm length

While there was no overall effect of temperature ($F_{3,97} = 2.10$, $p = 0.11$), trend analysis revealed a significantly positive linear relationship between temperature and sperm length ($F_{1,97} = 5.7$, $p = 0.019$): higher temperatures resulted in longer sperm (Fig. 1c left and Table 3). Family of origin influenced sperm length ($F_{15,97} = 4.08$, $p < 0.001$), whereas adult food level ($F_{1,97} = 2.45$, $p = 0.12$), tibia length (partial $r = 0.06$, $t_{97} = 0.68$, $p = 0.50$) and testis length

(partial $r = -0.02$, $t_{97} = -0.19$, $p = 0.85$) did not. The latter result again indicates no trade-off between sperm length and testis length within treatment combinations. There was also no such trade-off when pooling all data (Fig. 2d; partial $r = -0.12$, $t_{116} = -1.28$, $p = 0.203$). SD in sperm length within males varied among families ($F_{15,97} = 2.06$, $p = 0.018$), decreased with adult food level ($F_{1,97} = 15.11$, $p < 0.001$), but was affected neither by temperature ($F_{1,97} = 1.68$, $p = 0.18$) nor by hind tibia or testis length (hind tibia length: partial $r = 0.05$, $t_{97} = 0.49$, $p = 0.63$; testis length: partial $r = -0.08$, $t_{97} = -0.73$, $p = 0.47$; Table 3).

Accuracy of sperm length measurement

Sperm length measurement proved accurate when we compared two replicate measurements (means based on 30 individual sperm) of eight males for each of the three experiments. The overall correlation between a measurer's first and second measurements is $r = 0.8$, and the overall repeatability $R = 0.65$. Sperm length varied among experiments/measurers ($F_{2,23} = 4.40$, $p = 0.025$; variance component = 51.33 (65.26%); cf. Fig. 1) and individual males ($F_{21,24} = 4.76$, $p < 0.001$; variance component = 17.90 (22.76%); error variance component = 9.42 (11.98%)). Note that because replicate measurements were not based on the same individual sperm, estimates contain within-male variation in sperm length. Note also that because each data set was measured by a different person, mean values ± SD are not directly comparable between experiments (Tables 1–3).

Discussion

Male yellow dung flies showed a number of interesting and robust patterns of plasticity in testis and sperm length when we varied the two key environmental factors food and temperature in two life history stages. (1) Both testis and sperm length varied among families, indicating heritable components (cf. Ward 2000 for sperm length). (2) Testis length, and thus presumably male reproductive investment, showed a hypoallometric but non-linear increase with body size, a repeatable result in our population (cf. Henseler, 1998). (3) Testis length decreased with increasing developmental temperatures, in accordance with Bergmann's rule extended to ectotherms (Atkinson and Sibly, 1997), but also showed a complex cubic relationship with holding temperatures after eclosion. (4) In both life stages sperm length increased or showed a negative quadratic relationship with temperature, definitely not following Bergmann's rule (cf. Blanckenhorn and Hellriegel, 2002). (5) The increase of variation in sperm length within males with increasing temperature before eclosion and decreasing

food availability after eclosion indicates that our extreme treatments indeed were stressful. (6) Nevertheless, we found no evidence of a trade-off across individuals between sperm length and testis length for either treatment. (7) Neither testis nor sperm length were affected by larval or adult food availability (i.e. dung or prey), despite strong effects of dung availability on body size (Blanckenhorn, 1998a). This suggests that investment into reproduction is less sensitive to food restriction than investment into growth.

Testis size

Males reared with limited food (i.e. at high larval densities) and at high temperatures before eclosion but held at favourable conditions while reaching maturity developed shorter testes and smaller body size. However, when statistically removing treatment effects on body size, only the effect of temperature on testis size remained significant (Fig. 1b inset), perhaps indicating that larvae are better adapted to compensate for food than temperature stress. Our findings contrast with those for a British population where larval densities did have effects on testis size (Stockley and Seal, 2001). This interesting discrepancy may be explained by the different ways of manipulating larval densities (see Materials and methods): their result could be a response to larval numbers or encounter rates and ours to the amount of food per se. This presupposes that larvae of this species, who apparently roam the whole volume of dung available, are able to differentiate between these two treatments. In any case, this example seems to underscore the value of true, within-species replication (Palmer, 2000).

The overall relationship between testis length and hind tibia length was hypoallometric (i.e. allometric exponent $b < 1$; cf. slope in Fig. 2a), indicating that body size increases faster than testis size and that smaller males have relatively larger and/or larger males have relatively smaller testes (cf. Stockley and Seal, 2001). However, this does by no means imply an adaptive response. Hypoallometric relationships are common in biology and may have a more fundamental phylogenetic or physiological origin (e.g. Schmidt-Nielsen, 1984; Reiss, 1989; Fairbairn, 1997; Simmons *et al*., 1999). Perhaps more informative is that in our study the allometric relationship between testis and hind tibia length increased with size (Fig. 2a). This non-linear relation may suggest a minimum testes size to be attained by small males, and for large males it fits well with field observations of increasing strength of sexual selection as males get larger (Jann *et al*., 2000). It would, therefore, be interesting to see whether a similar pattern is also evident in a scatterplot of hind tibia and testis length for the Stockley and Seal (2001) data.

As sperm are continuously produced during adulthood, testes are expected to increase in size as they fill, at least during the pre-reproductive period right

after emergence (Foster, 1967; Ward and Simmons, 1991). We therefore also investigated the effects of temperature and food availability during the first 2 weeks of adulthood. Perhaps surprisingly, testis length showed an overall flat but significantly cubic relationship with increasing adult temperatures (Fig. 1c). This effect was independent of body size, as all males developed under the same favourable pre-adult conditions and therefore belonged to the same, largest size class (see Fig. 2c). Again, testis size was unaffected by prey availability, although similarly low food levels resulted in dramatically reduced clutch sizes (Jann and Ward, 1999). On the other hand, we found an almost significant interaction between our food and temperature treatments: an overall increase in testis length with warmer temperatures apparently only occurred when prey was abundant, perhaps because these conditions accelerate sperm development. Deprivation of prey for 8 days after emergence, however, does result in smaller testes (Foster, 1967). It also reduces the corpora allata, which seem to control both development of accessory cells surrounding the ejaculatory duct and sexual behaviour. Consequently, although spermiogenesis is not prevented, prey-deprived males do not copulate (Foster, 1967).

Sperm length

Our sperm length measurements were accurate and repeatable. Nevertheless, mean sperm length varied considerably among experiments (cf. Fig. 1). This may relate to genetic variation among data sets (Ward, 1998, 2000), but also to differences between the three measurers. Comparisons among (as opposed to within) the experiments are therefore limited. Like Gage and Cook (1994), we measured total cell length (see also Morrow and Gage, 2001c). This precludes inferences about potential separate responses of head and tail length, which are not correlated (Ward and Hauschteck-Jungen, 1993) and might vary independently.

Sperm length surprisingly was affected by temperature only and not by larval or adult food availability. In females the same temperature and food conditions after emergence showed clear effects on egg volume (Blanckenhorn, 2000). Sperm length was also not correlated with body size, thus confirming that these two traits vary independently (Ward and Hauschteck-Jungen, 1993; Hellriegel and Bernasconi, 2000). At the same time sperm length varied among families, supporting the previously found high heritability of sperm length in this species (Ward 2000; see also Ward, 1998).

In both life stages sperm length either increased (before and after eclosion) or first increased and then slightly decreased (before eclosion), but it certainly did not significantly decrease with increasing temperature like body and testis size. This suggests strongly that sperm cells do not follow the extended Bergmann's rule (Blanckenhorn and Hellriegel, 2002), which generally applies to

eggs and body cells (e.g. Partridge *et al.*, 1994; Azevedo *et al.*, 1996; Van Voorhies, 1996). Snook (2001) also did not find the expected latitudinal trend in sperm length in *D. subobscura*, despite a clear increase in wing length with increasing latitude (Huey *et al.*, 2000). Interpreting our result is difficult because almost nothing is known about sperm physiology in *S. stercoraria* and whether there is an adaptive optimum for sperm size. The significant negative quadratic relationship between sperm length and temperature (Fig. 1b) may suggest such an optimum at larger sperm sizes being achieved at favourable intermediate temperatures. The little or non-existing shortening of sperm with food or temperature stress allows two interpretations: either long sperm are not more costly to produce, or they confer some kind of advantage compensating for any such costs. While direct evidence for either of these conclusions is lacking in this species, producing the extremely long sperm of *Drosophila* proved to be costly (Pitnick *et al.*, 1995; Pitnick 1996).

High temperatures, but not larval food limitation, increased within-male variation in sperm length. Provided this variation reflects a male's ability to produce sperm of reliable length, our result indicates temperature stress. It agrees with corresponding increases in fluctuating asymmetry (i.e. small random deviations from symmetry in paired, bilateral traits) of legs and wings at high temperatures (Hosken *et al.*, 2000). In adults, in contrast, food limitation increased sperm length variation, but not high temperatures. Interestingly, stress susceptibility indicated by variation in sperm length seems heritable and, in one of our pre-adult experiments, was also significantly correlated with body size: larger males produced more variable sperm. It appears that within-male variation in sperm length is a sensitive indicator of genetic and environmental conditions in *S. stercoraria* (cf. Hoffmann and Woods, 2001).

Trade-off between sperm length and sperm numbers (testis size)

We found no direct trade-off between sperm size and numbers across males within treatment combinations, nor when pooling all males as if they were a single phenotypic sample. This assumes that testis size is a reliable measure of maximum sperm numbers, a standard assumption in many sperm competition studies (e.g. Simmons *et al.*, 1999; Hosken and Ward, 2001; Stockley and Seal, 2001). Similarly, Gage and Cook (1994) found no trade-off in the meal moth *Plodia interpunctella*, even though they estimated sperm numbers more directly. In their study, larval food shortage resulted in lower sperm numbers but left sperm size unaltered (Gage and Cook, 1994). In *S. stercoraria*, however, larval food availability did not significantly affect testis size.

On the other hand, we found an association between low temperature, large body size, short sperm and large testes which could be suggestive of a size-number trade-off. Predictably low temperatures during pre-adult development

are experienced by flies destined to overwinter as pupae in late autumn (Blanckenhorn, 1998a). As winter has a synchronizing effect, these flies will emerge at high densities, and males will experience a highly male biased operational sex ratio when they arrive at their mating sites, the dung pats (Jann *et al.*, 2000). Under these conditions being large and having large testes (i.e. high maximum sperm numbers) will definitely confer an advantage.

Conclusion

The missing effect of larval or adult food stress on testis and sperm length in *S. stercoraria*, despite strong effects of larval food on body size, suggests that investment into reproduction is less sensitive to limited resources than investment into growth. In this respect males clearly differ from females. The non-existing direct trade-off between sperm and testis length is in line with the theoretical prediction that sperm size optimization is independent of sperm competition risk (Parker, 1993). Surprisingly, sperm do not seem to show the same sensitivities to temperature as eggs (Blanckenhorn, 2002). This either questions that Bergmann's rule can be explained by a physiological mechanism (or constraint) or suggests that sperm differ fundamentally in their physiology from other cells (cf. Blanckenhorn and Hellriegel, 2002). These general conclusions may also hold for other species. They emphasize the need for more studies of environmental effects on sperm production and of the functional significance of sperm length, including artificial selection on sperm length (Morrow and Gage, 2001c) and sperm competition experiments between males with shorter and longer sperm (Morrow and Gage, 2001b).

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